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NEW RESEARCH HORIZON *Review*

Sperm competition and the evolution of spermatogenesis

Steven A. Ramm[1,*], Lukas Schärer[2], Jens Ehmcke[3], and Joachim Wistuba[4]

[1]Evolutionary Biology, Bielefeld University, Morgenbreede 45, 33615 Bielefeld, Germany [2]Evolutionary Biology, Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland [3]Central Animal Facility of the Faculty of Medicine, University of Münster, Albert-Schweitzer-Campus 1 (A8), 48149 Münster, Germany [4]Institute of Reproductive and Regenerative Biology, Centre of Reproductive Medicine and Andrology, University of Münster, Albert-Schweitzer-Campus 1 (D11), 48149 Münster, Germany

*Correspondence address. E-mail: steven.ramm@uni-bielefeld.de

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ABSTRACT: Spermatogenesis is a long and complex process that, despite the shared overall goal of producing the male gamete, displays striking amounts of interspecific diversity. In this review, we argue that sperm competition has been an important selection pressure acting on multiple aspects of spermatogenesis, causing variation in the number and morphology of sperm produced, and in the molecular and cellular processes by which this happens. We begin by reviewing the basic biology of spermatogenesis in some of the main animal model systems to illustrate this diversity, and then ask to what extent this variation arises from the evolutionary forces acting on spermatogenesis, most notably sperm competition. We explore five specific aspects of spermatogenesis from an evolutionary perspective, namely: (i) interspecific diversity in the number and morphology of sperm produced; (ii) the testicular organizations and stem cell systems used to produce them; (iii) the large number and high evolutionary rate of genes underpinning spermatogenesis; (iv) the repression of transcription during spermiogenesis and its link to the potential for haploid selection; and (v) the phenomenon of selection acting at the level of the germline. Overall we conclude that adopting an evolutionary perspective can shed light on many otherwise opaque features of spermatogenesis, and help to explain the diversity of ways in which males of different species perform this fundamentally important process.

Key words: evolution / spermatogenesis / spermatogonial stem cells / sperm competition / testicular function

Introduction

At some level, all sperm aim to do the same thing: fertilize an egg and contribute the paternal half of the resulting zygote's genome. Why then does sperm morphology differ so much across the Animal Kingdom, with the male gamete often being considered the most diverse animal cell type known (Pitnick *et al.*, 2009a)? Similarly, each egg only requires one sperm to fertilize it, so why then do males produce sperm in such prodigious quantities, generally far outnumbering the number of ova produced by females? The answers lie in understanding the evolutionary force of sperm competition and the profound effect it exerts on male reproductive biology. In fact, we will argue in this review that thinking about spermatogenesis in such an evolutionary framework holds great potential for explaining these and many other aspects of this most fundamental of male reproductive traits.

But first, what exactly is sperm competition? And why might it be so important? Put simply, sperm competition occurs whenever sperm from two or more males compete to fertilize a female's eggs (Parker, 1970, 1998). Until quite recently, it was assumed that multiple mating by females—and hence sperm competition—was relatively rare in nature, but the advent of DNA fingerprinting technology and other advances have drastically altered that view (see Zeh and Zeh, 2003). Among evolutionary biologists, sperm competition is now widely recognized as a pervasive influence on male reproduction (Birkhead and Møller, 1998; Birkhead *et al.*, 2009).

How then might sperm competition affect spermatogenesis? To begin with one major aspect of diversity between species—the numbers of sperm produced by the testis—let us compare two closely related species that differ in levels of sperm competition. Chimpanzees (*Pan troglodytes*) live in large multi-male, multi-female groups, and females typically mate with multiple males. By contrast, the gorilla (*Gorilla gorilla*) mating system is characterized by a single breeding male who monopolizes access to multiple females, thereby ensuring that his sperm do not have to compete with those from potential rivals. How would we predict these two mating systems to play out in terms of the investment males should make in sperm production? Presumably in the former case the sperm from one chimpanzee male faces competition from sperm from other males over which of them fertilizes the egg. Since the outcome of sperm competition likely often resembles a lottery in which buying more tickets than your competitors increases the chances of winning, this leads to an arms race over sperm numbers and an overall increase in male investment towards sperm production. Just as predicted,

and in spite of their much smaller body size, chimpanzee testes are almost four times heavier (in absolute terms) than those of gorillas, enabling the production of far greater numbers of sperm (Fig. 1; Short, 1979; Harcourt *et al.*, 1981; see also Wistuba *et al.*, 2003; Luetjens *et al.*, 2005).

To generalize, in evolutionary terms we can say that sperm competition exerts a special type of selection on males that is known as sexual selection (Darwin, 1871).This is simply the process by which any trait that increases the reproductive success of its bearer (relative to that of other individuals in the population)—and is at the same time heritable—will tend to be selectively favoured (i.e. alleles that positively affect that trait will tend to increase in frequency in the population). Individuals with an advantageous trait will tend to leave more offspring, and those offspring themselves will tend to express the same trait. So, in the recent evolutionary history of chimpanzees, the males making larger numbers of sperm must have gained greater reproductive success than their rivals because they did better in sperm competition, and the tendency to produce larger numbers of sperm was inherited in their offspring. Gorillas producing greater than average numbers of sperm likely gained no such reproductive advantage, because there were usually no rival sperm to outcompete, and in this species available resources would have been better channelled into other traits that can increase male reproductive success, such as fighting ability. This pattern of selection for increased sperm production in lineages subject to higher levels of sperm competition is repeated in numerous animal groups across a wide taxonomic range (e.g. Parker *et al.*, 1997; Hosken and Ward, 2001; Byrne *et al.*, 2002; Pitcher *et al.*, 2005; Ramm *et al.*, 2005), and is therefore undoubtedly of major evolutionary importance.

In what follows, we explore the general premise that thinking about sperm competition can help us understand not just selection on sperm numbers (and thus testis size), but also many other aspects of spermatogenesis. To do so, we have organized the review in two parts. In the next section, we briefly survey spermatogenesis in some of the main animal model species, to illustrate the wide interspecific diversity in the way that males produce their gametes. Although some of the gross differences we describe undoubtedly reflect a strong degree of historical (phylogenetic) contingency, we emphasize that there is a major question to answer about how and why much of this diversity has arisen. In the second part of the review, we then examine to what extent sperm competition could be the relevant factor to understanding diversity in spermatogenesis, within the confines imposed by the different broad testicular arrangements that we have described. We focus our discussion on a few particularly interesting aspects: (i) the number and morphology of sperm produced; (ii) the testicular organizations and stem cell systems used to produce them; (iii) the large number and high evolutionary rate of genes underpinning spermatogenesis; (iv) the repression of transcription during spermiogenesis and its link to the potential for haploid selection; and (v) the phenomenon of selection acting at the level of the germline. Our general aim is to point out that an appreciation of the evolutionary forces acting on male reproductive biology—and especially selection pressure due to sperm competition—can serve as an organizing framework to help us better understand the functioning of the testis and the process of spermatogenesis this organ supports.

Diversity of spermatogenesis

Spermatogenesis has to achieve three main aims: mitotic multiplication of the spermatogonial stem cell (SSC) population; meiotic recombination; and differentiation and maturation of spermatozoa. A balance must therefore be struck between the SSCs (i) producing differentiating daughter cells to meet current sperm production demand and (ii) maintaining a pool of undifferentiated SSCs by self-renewal and thus retaining the ability to produce sperm in the future. As we briefly illustrate in the following by describing spermatogenesis in a few established animal model systems, the way in which this process is organized to achieve this balance differs markedly between species, depending upon both their evolutionary history and the current ecological context (see also Roosen-Runge, 1977; White-Cooper *et al.*, 2009; Ramm and Schärer, 2014). An important common feature we emphasize here, however, is that replication errors during gametogenesis lead to novel, heritable mutations. Given that the number of cell divisions in spermatogenesis usually far exceeds that in oogenesis, this may make spermatogenesis one of the major sources of genetic novelty and adaptation (Li *et al.*, 2002; Ellegren,

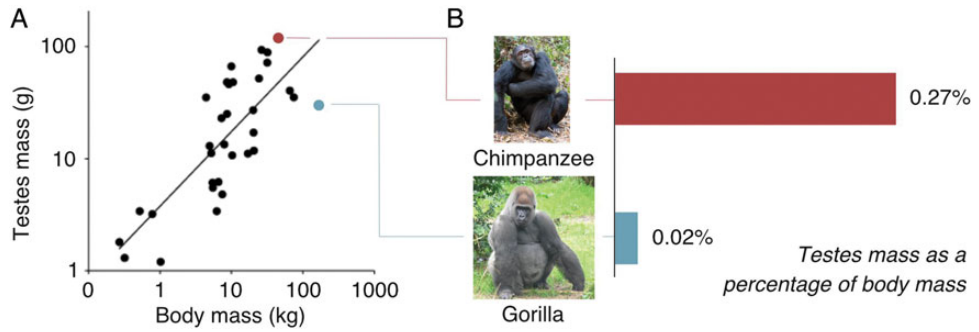

Figure 1 Testis size evolution in primates. In (**A**) data from 33 different species of primates are re-plotted from Harcourt *et al.* (1981) to show the allometric scaling of testis size (*y*) with body size (*x*): as might be expected, larger species tend to have larger testes (note that both axes are log-transformed). However, some species deviate substantially from the regression line, as is the case for the species pair depicted in (**B**), the chimpanzee (*Pan troglodytes*, plotted in red) and the gorilla (*Gorilla gorilla*, plotted in blue). The positive residual value for the chimpanzee (greater testis mass than expected for its body mass) and the negative residual value for the gorilla (lower testis mass than expected for its body mass) reflect the far greater importance of sperm competition—selecting for increased sperm production—in the former compared with the latter lineage. [Images: (http://commons.wikimedia.org/wiki/File:Gombe_Stream_NP_Alphatier.jpg) and (http://commons.wikimedia.org/wiki/Gorilla#mediaviewer/File:Male_silverback_Gorilla.JPG) Wikimedia Commons, licensed under GNU Free Documentation License v1.2 and CC-BY-SA-3.0 respectively.].

2007). Variation in spermatogenesis parameters may therefore have important evolutionary consequences far beyond the immediate context of reproduction. The patterns of strong selection on male reproductive traits and the large number of cell divisions involved in spermatogenesis, for example, carry with them a significantly elevated risk of developing cancer (Crespi and Summers, 2005; Kleene, 2005; Lewis *et al.*, 2008) and of the introduction of (usually deleterious) novel mutations. But such risks may nevertheless be tolerated precisely because of the immediate need to maintain high levels of sperm production under sperm competition (Blumenstiel, 2007).

Caenorhabditis elegans

Most individuals of the nematode *C. elegans* are protandrous sequential hermaphrodites, meaning that they first produce sperm and later eggs from a single gonad (allowing self-fertilization) (reviewed in Ward *et al.*, 1981; L'Hernault, 2006). Both the ca. 160 sperm produced from the fourth larval stage until adulthood, as well as all subsequently produced oocytes, arise from a single pool of precursors. The switch from sperm to oocyte production depends on a regulatory gene hierarchy with oogenesis being induced by the repression of *fem-3* expression. In the rare male individuals in the population, *tra-2* is inactivated, causing expression of *fem* and *fog* genes and resulting in a testis that produces only male gametes. In these males the gonadal tissue initially extends in an anterior direction from a distal tip, loops and bends back posteriorly. Here the gonad is connected to the cloaca by the *vas deferens*. The SSCs are localized at the distal tip and give rise to spermatogonia. Along the gonadal lobe, development takes place in a linear fashion; differentiating germ cells are attached to the rachis, a central core cytoplasm that supports the germ cells structurally and nutritionally. Mitotic propagation of the spermatogonia results in syncytial primary spermatocytes that enter meiosis. Passing the pachytene stage, the spermatocytes separate from the rachis and once meiosis is complete the haploid cells begin spermiogenesis. Lacking acrosome and flagellum, the resulting gametes move using their pseudopodium, and sperm derived from males is competitively superior to that derived from hermaphrodites (Singson *et al.*, 1999).

Drosophila melanogaster

In male flies the embryonic gonad is similar to the female gonad but gene expression patterns within the somatic gonadal precursor cells are different and induce the formation of the testis. Gonadal development and in parallel spermatogenic processes start during the larval period and pupation (reviewed in Fuller, 1993). The testis extends from an ovoid lobe into the adult organ: a coiled blind tube that opens into the seminal vesicle and the ejaculatory duct (Erickson and Quintero, 2007; Hime *et al.*, 2007). At the apical tip of the testis, attached to the basal lamina of the testis wall, the germinal proliferation centre is located, consisting of three cell types. Densely arranged apical cells form the conus of the central hub. Around this structure in close contact to the hub cells, the germ line stem (or pole) cells representing *Drosophila*'s SSCs are situated; each of these is enclosed by a pair of cyst progenitor cells (CPCs). The two somatic cell types (CPCs and hub cells) are in close contact via cytoplasmic extensions. Up to the third larval instar 16–18 SSCs are present and this number drops down to, and is maintained at, 5–9 SSCs in post-eclosion adults.

SSCs give rise to primary spermatogonia by mitotic division. The daughter cell that remains attached to the hub core remains as an SSC whilst the daughter that is displaced laterally enters into differentiation. Such a primary spermatogonium is enclosed in between two cyst cells that derive from the CPCs surrounding the SSC. The cyst cells do not divide further but stretch to enclose the further dividing germ cells (Fuller, 1993; Hime *et al.*, 2007). Therefore the cyst cells resemble a feature of the mammalian Sertoli cells, which also after an initial phase of propagation are terminally differentiated (see below). The two cyst cells and all the germ cells they enclose form a cyst, the unit of spermatogenesis. All germ cells in one cyst are derived from a single spermatogonium and thus represent a clone. In *D. melanogaster* each spermatogonium is thought to undergo four mitotic divisions with incomplete cytokinesis, resulting in a syncytium of 16 spermatocytes (but note that different *Drosophila* species exhibit strikingly variable numbers of mitotic divisions both within and between species, Schärer *et al.*, 2008). The connections between the germ cells are maintained, with each post-meiotic syncytial germ cell clone comprising 64 haploid early spermatids (but again see Schärer *et al.*, 2008 for deviations from that pattern). Starting spermiogenesis, the 'onion stage' is formed (Fuller, 1993) in which the numerous mitochondria of the differentiating germ cells fuse and form two mitochondrial derivatives including densely packed multi-layered membranes, serving as reserve material for the extreme elongation of the flagellum. During elongation the flagellar axonemes are assembled and the DNA condenses. At the end of spermiogenesis, the cytoplasmic bridges in the bundle of elongated spermatids are lost and the spermatozoa become individual cells. The extraordinarily long sperm (1.8 mm in *D. melanogaster*, but as long as 58 mm in *D. bifurca*; Pitnick *et al.*, 1995) are now coiled and afterwards they are released as coils from the cyst into the testis lumen. They reach the seminal vesicles, probably by endogenous motility, where they are stored until mating.

Fishes and amphibia

In general among vertebrates, male germ cells develop and differentiate in cohorts. The testes are organized in either of two general types: cystic or tubular (Fig. 2). Cystic testes are present, for example, in sharks and rays (Elasmobranchii) and in newts (Urodela) (Blüm, 1985). The seminiferous epithelium containing Sertoli cells and all germ cells form cystic structures referred to as spermatogenic ampullae or spermatogenic cysts, which to some extent resemble the testicular anatomy of insects. Each cyst contains an interconnected clone of germ cells that undergo differentiation in complete synchrony. Each gonad consists of a large number of these cysts: those at the start of development contain rather undifferentiated germ cells, such as undifferentiated spermatogonia, whereas cysts that have gone through further development have expanded to contain, in sequence, differentiating spermatogonia, spermatocytes and finally spermatids. The cysts are sequentially arranged throughout the lobes up to the tip of the testis where cysts contain mature sperm (Schlatt and Ehmcke, 2014). In such a cystic arrangement the number of premeiotic germ cell divisions essentially determines the size of germ cell cysts, which grow in size according to the number of germ cells they contain; assuming that no germ cells die while undergoing differentiation, an additional premeiotic division automatically leads to a doubling of the final cell number in the synchronized germ cell cohorts, and thus usually also to an increase in the final size of the cyst (although given the observations we mentioned above for *Drosophila* this may warrant some further investigation). Spermatogenic cyst size can differ greatly between species.

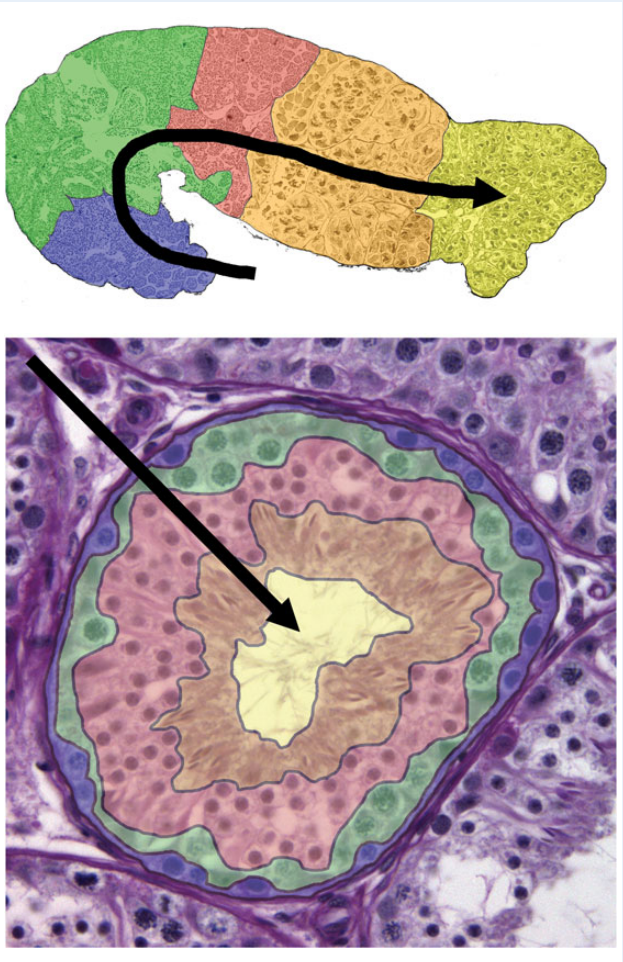

Figure 2 Schematic representation of the arrangement of germ cells in testes with either a cystic (upper image; Mexican Axolotl, *Ambystoma mexicanum*) or epithelial (lower image, human, *Homo sapiens*) form of spermatogenesis. Blue: spermatogonia; Green: spermatocytes; Red: round spermatids; Orange: elongated spermatids; Yellow: sperm.

In teleost fishes the cystic testis takes on a lobular or tubular organization (Chaves-Pozo *et al.*, 2005; Schulz *et al.*, 2010; Nakamura *et al.*, 2011), wherein the cysts are contained within elongated structures, called lobules or tubules. In the former, the cysts start developing at the blind end of the lobule and migrate towards the efferent system, and in the latter the spermatocysts are located along the tubule's basement membrane, and do not migrate (for a detailed description, see Loir *et al.*, 1995). Note, however, that this structure differs considerably from the structures usually referred to as 'seminiferous tubules' that are present in other vertebrates. As evidence for the wide variation in cyst size, one survey of cichlids found that the number of rounds of mitosis ranged from 8 to 10 in *Astatotilapia flaviijosefii* up to 16 in *Tilapia zillii* (Fishelson, 2003).

Reptiles, birds and mammals

In reptiles, the testicular anatomy is genuinely tubular (Blüm, 1985), but no recent studies have addressed the male reproductive biology in detail and so in the following we focus on describing spermatogenesis in mammals and birds (reviewed in Roosen-Runge, 1977; Kerr *et al.*, 2006). In both of these groups, spermatogenesis takes place in a lobular testis consisting of seminiferous tubules surrounded by interstitial tissue that is responsible for blood supply, immunological responses and steroidogenesis (by Leydig cells). The tubules are shaped by a basal lamina produced and covered by epithelial peritubular cells. These cells are myoid and drive the peristalsis necessary to transport the non-motile elongated testicular spermatozoa released from the apical seminiferous epithelium. Polarized Sertoli cells are attached to the inner side of the basal lamina, provide the attached germ cells with structural and nutritive support and mediate androgenic signals from the outside into the propagating germ line. Germ cell differentiation takes place from the basal lamina, in which the undifferentiated spermatogonia are embedded in supporting Sertoli cells. The spermatogonia undergo division both to renew themselves and to give rise to differentiating daughter cells that become spermatocytes, enter meiosis and then differentiate and elongate as haploid spermatids before they are released into the lumen of the tubules as mature sperm. One important variable parameter between species is Sertoli cell number, which stays constant in the adult after a prepubertal phase of proliferation, and determines the final testis size because each Sertoli cell can only support a limited number of germ cells ('Sertoli cell work load'; Wistuba *et al.*, 2007).

The most comprehensive and comparative studies of spermatogenesis have been performed in mammals. These have revealed that, in general, the process is quite similar across different mammalian species (Kerr *et al.*, 2006; Wistuba *et al.*, 2007). Crucial interspecific differences exist however in spermatogonial physiology (Ehmcke *et al.*, 2006). In primates, for example, there are two separate cell types of undifferentiated spermatogonia: the A_{dark} spermatogonia represent the mitotically inactive reserve stem cells (which only start to proliferate upon severe testicular damage, Ehmcke *et al.*, 2006), whereas the A_{pale} spermatogonia represent a type of mitotically active progenitor cell (Ehmcke and Schlatt, 2006). The differentiating germ cells derived from the A_{pale} population produce only ~128 sperm in the rhesus monkey, and 16 sperm in man (Fig. 3) (Ehmcke *et al.*, 2006). By contrast, in rodents several generations of A-type spermatogonia and of proliferating A-type spermatogonia exist; however, until recently only the so-called A_{single} spermatogonia have been considered to be the SSCs (but see Yoshida, 2012 and Hara *et al.*, 2014 for an alternative model).

Mammalian spermatogenesis requires a highly organized seminiferous epithelium. It is characterized by specific germ cell associations that arise from the topographic relationships of the different germ cell types. These associations, designated stages of spermatogenesis, can be determined histologically in cross sections of seminiferous tubules. In most mammals analysed so far, one single, specific stage fills the complete circular epithelial space (single-staged). When different germ cell associations are present simultaneously, this arrangement is characterized as multi-stage organization, a pattern that appears to occur more regularly in primates (Luetjens *et al.*, 2005).

In birds, many studies have been conducted on sperm morphology and sperm competition (see, e.g. Birkhead, 1998) but relatively few studies are available on the diversity of spermatogenesis. Various types of spermatogenic organization have been reported (e.g. multi- versus single-staged arrangements of the seminiferous epithelium, Lüpold *et al.*, 2011), but there is almost nothing known about the SSC systems apart from some domestic species in which these cells were investigated to find possible routes for transgenesis (e.g. Kalina *et al.*, 2007; Yu *et al.*, 2010). One relevant

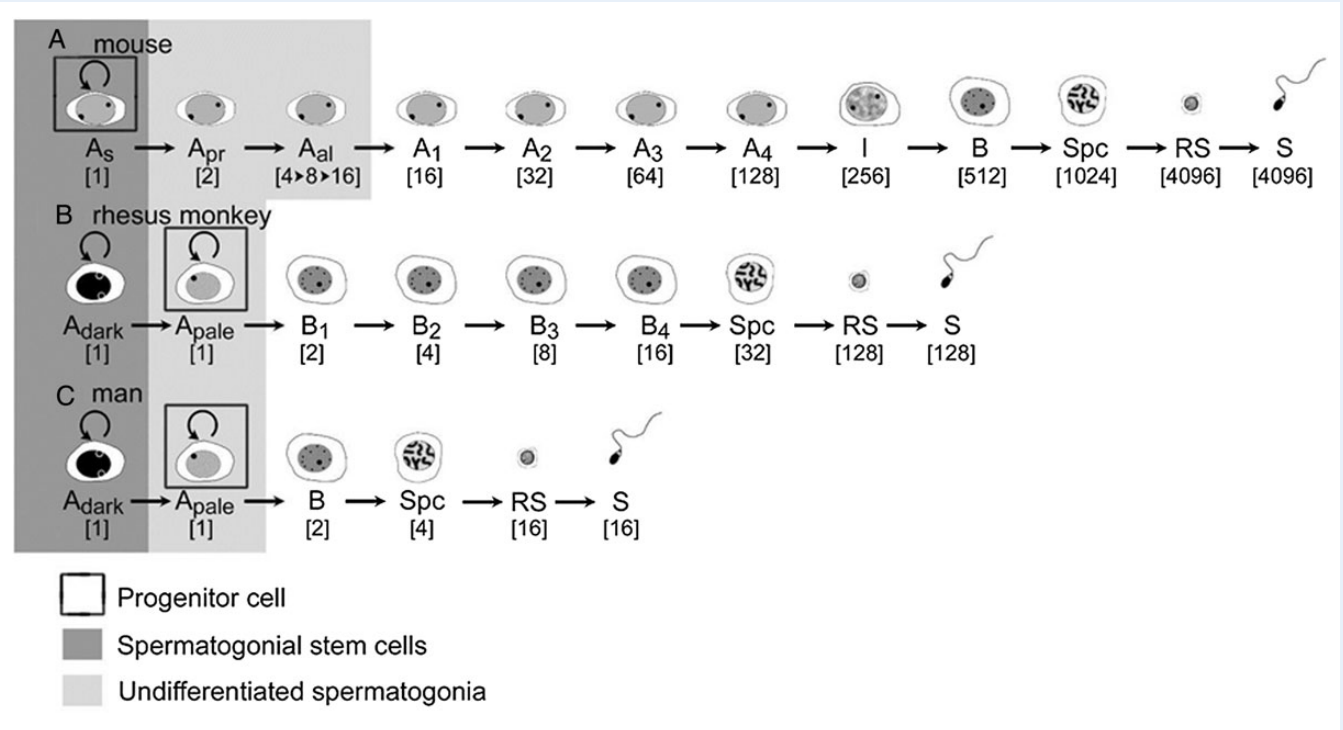

Figure 3 Schematic depiction of male germ cell differentiation in (**A**) the mouse, (**B**) the rhesus monkey and (**C**) man. Spermatogonial subtypes: As: A_{single}; Apr: A_{pair}; Aal: $A_{aligned}$; I: intermediate; B: B-spermatogonium. Spc: spermatocyte; RS: round spermatid; S: sperm. Note the very different number of sperm produced per spematogonial stem cell division in these three different species (Modified from Ehmcke *et al.*, 2006).

factor in most birds is that the location of the testis may be constrained by the need to fly (and similarly in some mammals by the need to be able to swim); precisely where in the body the testis is located could have important consequences for its function (though see Lovegrove (2014) for current debate over why and how many mammalian lineages exhibit a scrotal testis).

Evolutionary forces shaping aspects of spermatogenesis

In the previous section, we briefly demonstrated how the way in which spermatogenesis is organized can vary from one clade to another, and pointing out that such variation exists is one major goal of this review. Nevertheless, the element of historical contingency in explaining the gross organization of spermatogenesis when one compares across major animal groups still leaves a great deal of diversity unexplained. In this section, we therefore next explore to what extent an evolutionary perspective informed by the selective pressure of sperm competition can potentially illuminate this discussion. We pointed out in the Introduction the strong impact of sperm competition on sperm production rate and thus overall testis size, and evolutionary biologists have in the past principally focused on this simple measure of male investment in sperm production as one major aspect of interspecific diversity. Other potentially adaptive features of spermatogenesis have largely been ignored (Ramm and Schärer, 2014). The different aspects we consider in the following are summarized in Table 1, and we also refer the reader to Ramm and Schärer (2014), which contains a more thorough treatment of some of these topics, as well as examining other aspects that space constraints prevent us from discussing here.

Sperm number and morphology

Since sperm competition often selects for males to produce more sperm, it seems reasonable that this will result in adaptations within the testis to increase the efficiency of spermatogenesis, which may go beyond simply increasing testis size. For example, it has recently been shown that the proportion of spermatogenic tissue contained within the testis increases in lineages subject to higher levels of sperm competition both in birds and mammals (Lüpold *et al.*, 2009; Rowe and Pruett-Jones, 2011; Montoto *et al.*, 2012; delBarco-Trillo *et al.*, 2013). Sperm competition also likely explains variation in the duration of spermatogenesis: high levels of sperm competition select for a shorter cycle length of the seminiferous epithelium in mammals, enabling more sperm to be produced per unit time (Ramm and Stockley, 2010), though this relationship appears to depend also on the type of sperm that must be produced (see below) and on the metabolic rate of the species in question (Ramm and Stockley, 2010; delBarco-Trillo *et al.*, 2013).

In many animal groups it is likely not just the number of sperm that are produced that is important for males to achieve fertilization success under sperm competition, but also their morphology (Pizzari and Parker, 2009; Lüpold *et al.*, 2012). Indeed, the fact that the sperm cell is the most diverse animal cell type known attests to the great importance of sperm morphology to reproductive fitness (Pitnick *et al.*, 2009a). Again, selection on sperm morphology is likely reflected in the way that spermatogenesis is organized (Ramm and Schärer, 2014), from

Table 1 Summary of different aspects of spermatogenesis discussed in this review and pertinent evolutionary considerations.

Aspect of spermatogenesis	Evolutionary considerations
Number of sperm produced	Affects likelihood of competitive fertilization success, with greater sperm production being strongly favoured under greater levels of sperm competition; this selects for more sperm-producing tissue and for greater spermatogenic efficiency
Morphology of sperm produced	Affects sperm performance in the external medium or female reproductive tract and thus fertilization success, and is thus strongly affected by sperm competition and cryptic female choice; different sperm morphologies have different spermatogenic requirements
Testicular organization and spermatogonial stem cell system	Several interrelated factors including sperm competition, reproductive lifespan, seasonality and mode of fertilization could all influence the way in which spermatogenesis is organized within the testis and the stem cell system used to support it
Genetics of spermatogenesis	The large number and rapid evolution of testis-specific genes is likely at least partially attributable to sperm competition
Repression of transcription and haploid selection	Between-ejaculate sperm competition selects for males that repress within-ejaculate sperm competition; sperm competition sometimes favours the evolution of sperm cooperation
Germline (selfish spermatogonial) selection	*De novo* mutations in spermatogonial cells can sometimes selfishly expand their representation within the spermatogonial stem cell population; even small advantages can accumulate, given the very large number of cell divisions (which is ultimately due to the evolution of anisogamy and sperm competition)

See 'Evolutionary forces shaping aspects of spermatogenesis' section of the main text for full details and references.

the correlated evolution of sperm length and testicular architecture (number of rounds of mitosis per spermatogonial stem cell division) in *Drosophila* (Schärer *et al.*, 2008) to the direct link between sperm size, dimensions of the seminiferous epithelium and spermatogenic efficiency in New World blackbirds (Lüpold *et al.*, 2009, 2011). Importantly, recent work also emphasizes the trade-offs between sperm size, number and performance that likely result from males investing a limited pool of reproductive resources into spermatogenesis (e.g. Schärer *et al.*, 2008; Ramm and Stockley, 2010; Immler *et al.*, 2011; Lüpold *et al.*, 2011; but see Fitzpatrick *et al.* 2009), meaning that to understand why spermatogenesis is organized in a particular way one must understand how selection is acting on sperm number, sperm size and other sperm traits relevant to sperm competition outcomes. A likely critical factor here is the 'battleground' in which ejaculates compete, i.e. the characteristics of either the external environment (in external fertilizers) or the female reproductive tract (in most internal fertilizers) where sperm competition is actually resolved (Pitnick *et al.*, 2009b; Immler *et al.*, 2011). Moreover, female influences on sperm competition outcomes—termed 'cryptic female choice'—should also not be ignored (Eberhard, 1996), nor should other potential sources of variation in sperm production parameters less directly linked to sperm competition, such as biased sex ratios (e.g. Reuter *et al.* 2008) or high mating rates (see Vahed and Parker, 2012).

Testicular and SSC organization

Changing patterns of overall sperm demand over evolutionary time may also help to understand more fundamental shifts in the way spermatogenesis is organized. Recall that in the comparison between the chimpanzee and gorilla testes (see Introduction) it was concluded that differing levels of sperm competition experienced by these two species had strongly influenced the evolution of their widely differing relative testis size. While this seems to be the primary response to sperm competition in primates (Wistuba *et al.*, 2003), these and other primate species also differ in several other aspects of spermatogenesis and the possible influence of mating system variation on these additional aspects is currently unclear (Wistuba *et al.*, 2003).

More broadly, it would not be surprising if other features of spermatogenesis and testicular development would also reflect requirements set by variable conditions from one species to another, though not necessarily directly linked to variation in sperm competition. For example, the balance between sperm-producing tissue and interstitium is completely different in birds compared with mammals, although the testicular organization is quite similar (Lüpold *et al.*, 2009).

Similarly, life history parameters, such as reproductive lifespan, or particular features of a species' reproductive biology likely also set important restrictions on the optimal way in which spermatogenesis should be organized. For example, an animal may have a short or a long reproductive lifespan, and sperm production can be a seasonal or a continuous process. As variation in reproductive lifespan and seasonality occurs amongst species with different types of testes, it is likely not the gross organization of the testicular tissue (lobular versus cystic) itself, but rather the spermatogenic processes occurring within it that might be most strongly affected by the evolutionary pressure originating from these differing demands (see also Ramm and Schärer, 2014).

Against this background, it might be worthwhile to consider the SSC systems as likely candidates for adaptation. Data on these are quite limited but a comparison between rodent and primate SSC systems reveals remarkable differences. Whilst in rodents many SSCs propagate simultaneously and all of them produce clones permanently, in primates a progenitor system is present with an active (A_{pale}) and a reserve stem cell (A_{dark}) population (Ehmcke *et al.*, 2006; Ehmcke and Schlatt, 2006; Wistuba *et al.*, 2007). The latter are only activated and recolonize the tubules when the testis undergoes a lesion (e.g. inflammation, infection) and thereby regain (or maintain) reproductive capacity. When comparing the postpubertal—i.e. the reproductive—lifespan of a mouse and a

macaque the likelihood to suffer from an event that disturbs spermatogenesis is much higher in the primate than it is in a mouse, which normally reproduces only for 1 year. Thus it could be speculated that the SSC systems have developed according to these requirements—a question that could be of interest not only to reproductive but also evolutionary biologists.

Given that the SSC systems of rodents and primates are remarkably different, it seems plausible to assume that the regulatory processes underlying their stem cell dynamics also differ. For preclinical research on male reproduction, non-human primates are therefore the model of choice as they exhibit the primate-specific progenitor buffered stem cell system, characterized by the maintenance of a spermatogonial balance between active (A_{pale}) and the inactive reserve (A_{dark}) cells. However, since a route has not yet been established for transgenic manipulation to understand genotype–phenotype relationships in non-human primates, it is clearly still necessary to employ mouse models to study the genetics underlying certain processes involved in spermatogenesis and male reproduction. As is the case for any research employing model organisms, one has to consider that results obtained from the phylogenetically rather distant rodent models may not be directly transferable to the human (clinical) context, especially as far as they concern the testicular stem cell function (Ehmcke *et al.* 2006).

Number and evolution of spermatogenesis genes

The molecular genetic details of spermatogenesis are understood in only a few model systems, most notably in *C. elegans*, *D. melanogaster* and *Mus musculus*, and their similarities and differences have been well described in an evolutionary context elsewhere (White-Cooper *et al.*, 2009; White-Cooper and Bausek, 2010). Here we focus on a few broad scale patterns of evolution for the class of testis-specific genes as a whole, which display some very interesting properties.

First, there are an extremely large number of testis/sperm-specific genes (e.g. Chintapalli *et al.*, 2007), and these often appear to substantially outnumber ovary/oocyte-specific genes (sometimes by up to an order of magnitude). This pattern has been described for example in *C. elegans* (Reinke *et al.*, 2000), *D. melanogaster* (Parisi *et al.*, 2004; but see Perry *et al.* 2014), zebrafish *Danio rerio* (Small *et al.*, 2009) and in the simultaneously hermaphroditic flatworm *Macrostomum lignano* (Arbore *et al.*, in preparation). One possible explanation for this could be arms races and strong selection for evolutionary novelty driven by sperm competition and related phenomena, and indeed there is some evidence that duplicated (e.g. Parsch *et al.*, 2001; Torgerson and Singh, 2004; Clark *et al.*, 2007; Baker *et al.*, 2012; Yeh *et al.*, 2012) and *de novo* genes (e.g. Levine *et al.*, 2006; Begun *et al.*, 2007; Heinen *et al.*, 2009) commonly have expression patterns or phenotypic effects consistent with roles in male reproduction and sperm competition. However, the pattern of more male-biased genes is not universal (e.g. more female-biased genes are found in the gonads of turkeys *Meleagris gallopavo*; Pointer *et al.*, 2013), and more work is needed to understand any potential links between patterns of gene origins, gene expression and the role of sperm competition and related factors such as sexual antagonism (Parsch and Ellegren, 2013).

A closely related question concerns the molecular evolution of testis-specific and other sex-biased genes, many of which show a heightened rate of sequence evolution consistent with positive Darwinian selection (e.g. Cutter and Ward, 2005; Haerty *et al.*, 2007). Again, it is plausible that such a pattern arises due to the strong selective pressures of sperm competition and related phenomena (Swanson and Vacquier, 2002; Parsch and Ellegren, 2013). An interesting pattern in this context comes from the study of Good and Nachman (2005), who compared rates of evolution of genes expressed during different stages of mouse spermatogenesis. They found that evolutionary rates correlate with developmental timing of expression, and that late-expressed genes, which presumably function during spermiogenesis, exhibit most signatures of positive Darwinian selection (Good and Nachman, 2005). This pattern also appears to extend beyond spermatogenesis, in that many genes whose products are important during later stages of the sperm 'lifespan'—most notably those involved in sperm-egg interactions—also exhibit signatures of positive selection (Dean *et al.*, 2008; Dorus *et al.*, 2010; Vicens *et al.*, 2014). The pattern identified by Good and Nachman (2005) is consistent with the idea that many of the species-specific phenotypes of most relevance to sperm competition develop during these later stages of spermatogenesis. Indeed, recent studies aimed at examining the evolution and expression of specific spermatogenesis genes putatively influencing sperm morphology—such as protamines and transition nuclear proteins—support this view (Lüke *et al.*, 2014a, b).

Repression of transcription and haploid selection

Two fundamentally different forms of sperm competition could in principle occur, either between sperm from the same ejaculate (i.e. intra-ejaculate competition) or between sperm from different ejaculates (i.e. inter-ejaculate competition). These two forces may act somewhat against each other: when a male's ejaculate must compete with sperm from other males over the fertilization of a female's eggs, there is a strong incentive for sperm function to be determined by the diploid paternal genotype, thus preventing the expression of the haploid sperm genotype via post-meiotic repression of transcription (Haig and Bergstrom, 1995). Moreover, inter-ejaculate sperm competition may potentially favour cooperative adaptations in sperm from the same male to maximize the male's reproductive success (reviewed in Immler, 2008; Pizzari and Foster, 2008; Higginson and Pitnick, 2011). This means that realized intra-ejaculate competition will normally be absent, and in fact the term 'sperm competition' is usually reserved for the inter-ejaculate phenomenon. Nevertheless, the potential for intra-ejaculate competition certainly exists (the next section being arguably one example), and there is growing evidence for post-meiotic transcription in sperm (Barreau *et al.*, 2008; Vibranovski *et al.*, 2010) and an increasing recognition of the potential importance of haploid selection (Joseph and Kirkpatrick, 2004). We can predict that the prevalence of intra-ejaculate conflicts such as meiotic drive alleles, which increase their transmission by gaining an unfair advantage in meiosis, will be directly and negatively correlated with the prevalence of sperm competition (Haig and Bergstrom, 1995; e.g. Price *et al.*, 2008; Manser *et al.*, 2011; Wedell, 2013). A recent model suggests that intra-ejaculate competition may also contribute to explaining patterns of rapid evolution of male reproductive genes referred to above (Ezawa and Innan, 2013), though how the presence of inter-ejaculate competition would affect the theoretical predictions is currently unclear.

Germline selection

The phenomenon of germline selection (i.e. selfish spermatogonial selection) likely explains a class of human 'paternal age effect' (PAE) mutations responsible for disorders including achondroplasia, Apert syndrome, Noonan syndrome, Costello syndrome and multiple endocrine neoplasia types 2A and 2B (Arnheim and Calabrese, 2009; Goriely and Wilkie, 2012; Maher *et al.*, 2014) caused by mutations in genes such as fibroblast growth factor receptor (FGFR) 2 (*FGFR2,* Goriely *et al.*, 2003; Qin *et al.*, 2007; Choi *et al.*, 2008), *FGFR3* (Lim *et al.*, 2012), the receptor tyrosine kinase proto-oncogene *RET* (Choi *et al.*, 2012), the RAS proto-oncogene Harvey rat sarcoma viral oncogene homolog (*HRAS*) (Giannoulatou *et al.*, 2013) and *PTPN11*, encoding tyrosine phosphatase non-receptor type 11 (Yoon *et al.*, 2013). The causative *de novo* point mutations are more prevalent in the testis than would be expected based on differences in the cell division number occurring during spermatogenesis compared with oogenesis alone, suggesting either that they occur at 'mutational hotspots' or that SSCs acquiring the mutation are somehow positively selected, i.e. gain a transmission advantage. Recent evidence points to the latter mechanism. Analyses using a variety of methods (see Goriely and Wilkie, 2012) have revealed that the allelic and spatial distribution of PAE mutations is most consistent with a model whereby certain point mutations confer a selective advantage on the SSCs harbouring them, leading to their localized clonal expansion and spread along the seminiferous tubule. In rare cases, this may progress to spermatocytic seminoma (Maher *et al.*, 2014). The selective advantage that these stem cells enjoy apparently derives from the fact that their causal mutations all occur at loci involved in the growth factor receptor-RAS signal transduction pathway (Goriely and Wilkie, 2012). Given the huge numbers of cell divisions that occur within the testis, there is a substantial opportunity for *de novo* mutations in key cellular pathways to occur. Even a small subsequent selective advantage can in the long term cause a major shift in the genetic composition of the testicular stem cell population. For example, it has been estimated that occasional symmetrical stem cell division (producing two new SSCs), for example once every 100 regular asymmetrical stem cell divisions, could suffice to generate the typical spatial distribution of PAE mutations, as could more complex but biologically more plausible variants on this basic idea (reviewed in Yoon *et al.*, 2013; Maher *et al.*, 2014). Of course, the large number of testicular stem cell divisions, which are responsible for the fact that selection at the level of the germline needs to be considered at all, ultimately links back to sperm competition and the evolution of anisogamy, i.e. the production by proto-males of many, smaller gametes and by proto-females of few, larger gametes (Lehtonen and Parker, 2014).

Conclusions and outlook

In this brief survey we have attempted to highlight how thinking about the selective pressures acting on spermatogenesis can be a useful framework for understanding how this important process is organized, and for explaining interspecific (and probably intraspecific) diversity in its details. In particular, although not often considered in the biomedical literature, we have demonstrated that sperm competition is a pervasive and influential evolutionary force acting on male reproductive biology, and a relevant factor for explaining multiple aspects of sperm production. As we have illustrated, these include some of the most important outstanding gaps in our knowledge about spermatogenesis, such as why different species vary so much in such basic features as the number and type of sperm produced, and in the stem cell systems that support this process. We suggest that fusing the proximate and ultimate outlooks traditionally adopted by reproductive and evolutionary biologists, respectively, holds great potential for further advancing our understanding of spermatogenesis, and hope that our review might help stimulate greater dialogue between researchers in these two traditionally quite separate fields. We see some cause for optimism on this front, in part enabled by vastly improved access to genetic and genomic data through next-generation sequencing and associated technologies, and by the increasing application of molecular techniques that were once the preserve of a small number of traditional model systems to a broader range of species. This breadth will often be needed to test particular evolutionary hypotheses derived from a 'sperm competition perspective'.

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Authors' roles

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Conflict of interest

None declared.

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